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Sex differences in *Drosophila* development and physiology

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Male and female flies differ in many aspects of development and physiology. Identifying the mechanism(s) underlying sex differences in cell and body growth, organ function, and metabolism is important in understanding how these male–female differences in development and physiology are created. Recently, studies in *Drosophila* have advanced our understanding of the sex-specific regulation of growth and cell signaling pathways, organ homeostasis, and metabolism. Here, we highlight how this knowledge provides important insight into the mechanisms underlying sex differences in body size, stress responses, lifespan, and disease processes. In addition, we will discuss how studying development and physiology has revealed previously unrecognized complexity in the *Drosophila* sex determination pathway.

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Introduction

Sex differences in *Drosophila* have been studied in exquisite detail for over 100 years. Males and females differ in sexual traits and reproduction (e.g. genitals, abdominal pigmentation, reproductive organs), and in development and physiology (e.g. body size, stress responses, lifespan). Enormous progress has been made in elucidating the mechanisms underlying sex differences in sexual traits, reproduction, development, and behavior [1–7]. The goal of this review is to highlight how recent studies on organ growth and function, growth regulation and body size, and adult metabolism and physiology, have further advanced our understanding of sex differences in multiple aspects of development and physiology. In addition, we will discuss how these recent studies on *Drosophila* development and physiology have provided new insights into the canonical sex determination pathway.

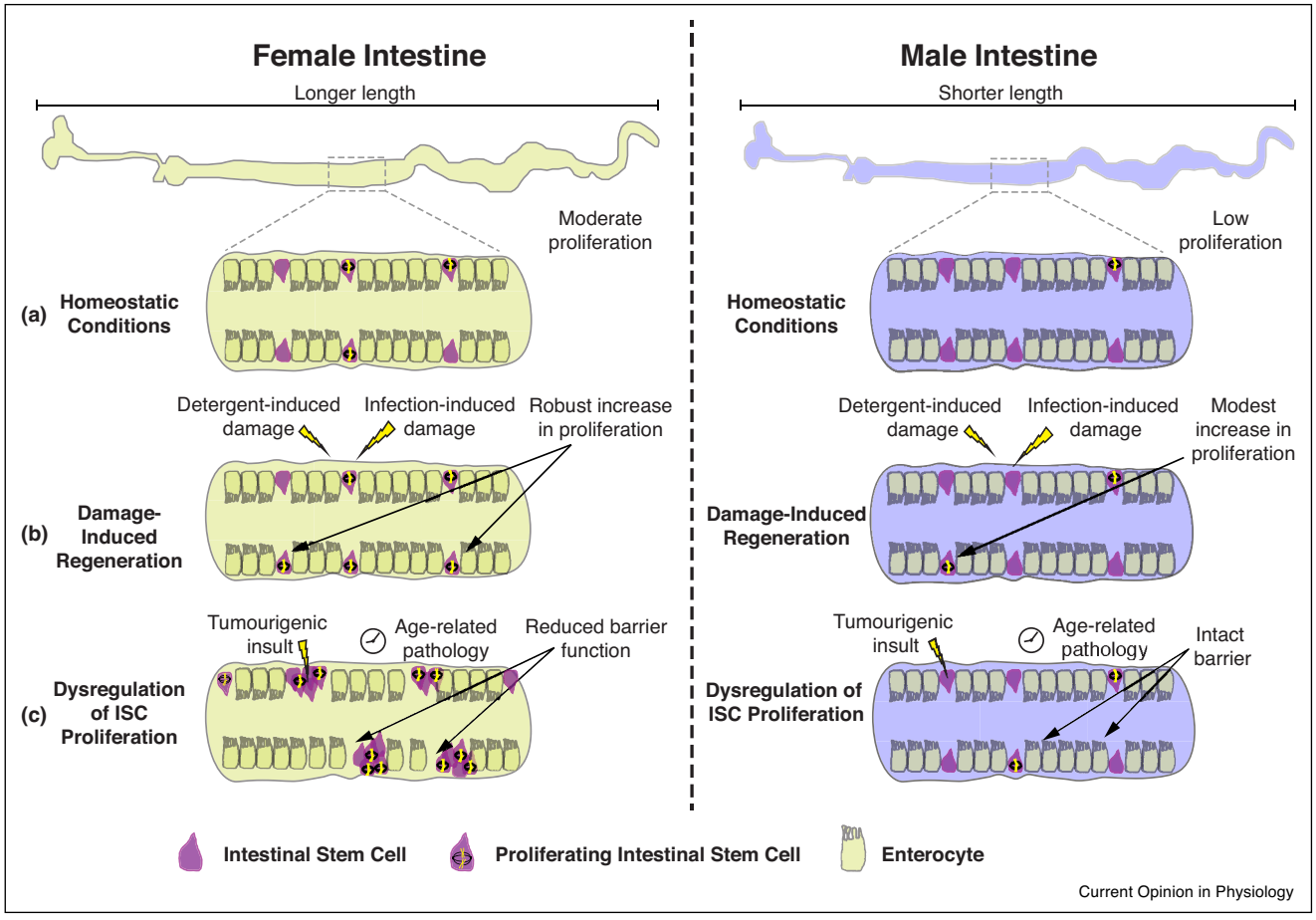
Sex differences in organ homeostasis: lessons from the *Drosophila* intestine

The mechanisms underlying male–female differences in sex-limited adult structures and sexually dimorphic organs (e.g. abdominal pigmentation, gonads) are well studied; however, less is known about sex differences in cells and organs without obvious sexual dimorphism. In this section, we will highlight recent studies on male–female differences in the *Drosophila* intestine (Figure 1), and discuss the implications of these findings for sex differences in the regulation and function of other organs.

In flies, the digestive tract breaks down food, absorbs nutrients, regulates energy homeostasis, provides a barrier against the external environment, and communicates with other organs [8]. Recently, many aspects of intestinal physiology were found to differ between male and female flies [9••,10•,11,12••], revealing previously unrecognized sex differences in this key organ. While many factors affect intestinal physiology, one determinant of intestinal growth and tissue integrity is the proliferation of intestinal stem cells (ISCs) [8,13]. When ISC proliferation was compared in males and females, significant sexual dimorphism was identified: ISC proliferation is higher in virgin females than in males [9••,12••]. This sex difference is further enhanced by mating, as ISC proliferation is significantly higher in mated females compared to virgin females [10•]. One recent study demonstrated a key role for juvenile hormone (JH) in promoting elevated ISC proliferation in mated females, and extended this finding by showing that JH was also responsible for mating-induced changes to lipid metabolism in differentiated enterocytes [10•]. Future studies in both the ISCs and enterocytes will be important to fully characterize sex differences and mating-induced changes to these cells, and to examine other intestinal cell types such as the enteroendocrine cells.

In addition to identifying sexual dimorphism and mating-induced changes to ISCs and enterocytes, recent studies have also provided significant insight into how these differences impact physiology in males and females throughout the lifespan. For example, increased gut length due to elevated ISC proliferation enhances reproductive output, as females with reduced gut length had a modest reduction in egg production [9••]. Similarly, the female-biased lifespan extension in response to dietary restriction and rapamycin feeding is associated with a female identity in the enterocytes of the mid-gut [12••].

Figure 1



Sex differences in intestinal growth, regeneration, and dysfunction. Recent studies identified significant sex differences in the proliferation of intestinal stem cells (ISCs) in the *Drosophila* gut in different contexts. **(a)** Under homeostatic conditions, female ISCs proliferate at a higher rate than male ISCs. This leads to increased gut length in females [9••]. **(b)** In response to infection-induced and detergent-induced damage to the intestinal epithelium, female ISCs maintain a higher rate of proliferation than male ISCs [9••,12••]. **(c)** Females develop increased age-related dysfunction of the intestinal epithelial barrier compared to males [12••], and have increased susceptibility to genetically induced tumors [9••].

In mated females, changes to the gut alter defecation frequency, fecal pH and water content [11], which could potentially affect nutrient absorption. Females also survive longer in response to infection-induced damage to the intestinal epithelium [12••]; however, more work is needed to determine whether this increased survival is linked to sex differences in ISC proliferation and enterocyte physiology. Despite these benefits, there are disadvantages associated with female-specific aspects of intestinal physiology; for example, increased ISC proliferation makes females more susceptible to age-induced gut barrier dysfunction [12••], and to the development of genetically induced tumors [9••]. Given the diverse traits associated with intestinal regulation and function, such as lifespan [14–16], nutrient absorption [8], and energy homeostasis [8,17,18], future studies will likely uncover more physiological consequences of sex differences in the intestine.

Beyond the intestine, these studies reveal a critical need for systematic studies on sex differences and mating-induced changes in other organs. Transcriptomic analysis has revealed sex differences in gene expression in many organs [19–23]; yet, the developmental and physiological consequences of these differences remain largely unknown. Expanding our knowledge of fundamental cellular processes and organ function in both males and females will provide critical insight into sex differences in physiology, stress responses, aging, and disease susceptibility.

Sex differences in body size: insights into the sex-specific regulation of signaling pathways

Adult female flies are significantly, and visibly, larger than male flies. Despite studies demonstrating important roles for cell–cell signaling pathways in the regulation of sex differences in imaginal disc growth [24–29], the signaling

pathways responsible for creating a male–female difference in larval and adult body size (sexual size dimorphism, SSD) remain unclear. In this section, we will describe recent advances in our knowledge of which signaling pathways may be involved in creating SSD in *Drosophila*, and discuss how this sex-specific regulation of cell signaling may impact other aspects of development and physiology.

Final body size in *Drosophila* is determined by the rate and the duration of larval growth [30]. Recent studies revealed that the mechanism underlying increased body size in females is an elevated rate of larval growth [31,32,33••]. In flies, the insulin/insulin-like growth factor signaling pathway (IIS) plays a key role in promoting an increased rate of larval growth in response to nutrient input [34–36], and temperature [37], where increased IIS activity stimulates growth to enhance body size [38–41]. Interestingly, IIS was recently implicated in the regulation of SSD, since SSD in adult weight was abolished in flies carrying hypomorphic mutations in the *insulin receptor* gene (*InR*) [32]. Supporting a role for IIS in regulating SSD, flies raised on low nutrient medium, which reduces IIS pathway activity, abolished SSD in pupal volume [42••], a measure of larval growth. This reduction in SSD is not simply a generalized effect of reducing growth in the faster-growing sex, since SSD is preserved in animals with pharmacological inhibition of the target of rapamycin (TOR) pathway [42••], another nutrient-responsive growth pathway that affects body size [43,44]. Taken together, these results support a key role for IIS in regulating SSD [32,42••].

An obvious line of enquiry arising from these studies is a comparison of IIS regulation in males and females. One important way that IIS activity and function are modulated is via regulation of the *Drosophila insulin-like peptides* (*dilps*). Although we currently lack a comprehensive examination of *dilp* regulation in male and female larvae, due to complex regulation of *dilp* genes [40,41,45–48], Dilp proteins [31,49–53], and Dilp secretion from the insulin-producing cells (IPCs) [50,54–58], recent progress has been made. For example, in late third instar larvae, *dilp3* transcript levels were found to be male-biased, whereas the secretion of Dilp2, an important growth-promoting Dilp released from the IPCs [40,41,54,59,60], was higher in female larvae [42••] (Figure 2). This difference in *dilp* regulation may affect IIS activity, as a comparison of IIS activity in late third instar larvae suggests IIS activity is higher in female larvae than in male larvae at this stage [42••], though not at earlier larval stages [33••,42••]. Yet whether the sex-specific regulation of *dilp3* and Dilp2, and possibly other *dilps*, affects SSD remains unresolved. In one study, the loss of *dilp2* had female-biased effects on adult weight (11% reduction in females, 5% reduction in males), whereas tandem loss of *dilp2,3* reduced adult weight in both sexes by 7% [45]. In a separate study, SSD in larval weight was unaffected by loss of *dilp2*, or loss of *dilp1-5* [33••]. At first glance, this data seems to argue against a role for the *dilps* in creating SSD; however, levels of *dilp5* mRNA are up-regulated in *dilp2,3* double mutants, and *dilp6* mRNA is strongly up-regulated in animals lacking *dilp2,3,5* [45]. Thus, increased knowledge of the sex-specific regulation of all *dilp* genes and Dilp proteins, including compensatory regulation [45,46], will be required to interpret data from studies investigating a role for the *dilp* genes in the regulation of SSD. Further, given that both *dilp* regulation and SSD are modulated by nutrient quantity and quality [41,61,62], identifying sex-by-diet interactions on Dilp regulation and SSD will be essential to understand the individual and combinatorial effects of mutations in *dilp* genes on SSD, and other IIS-regulated phenotypes such as lifespan.

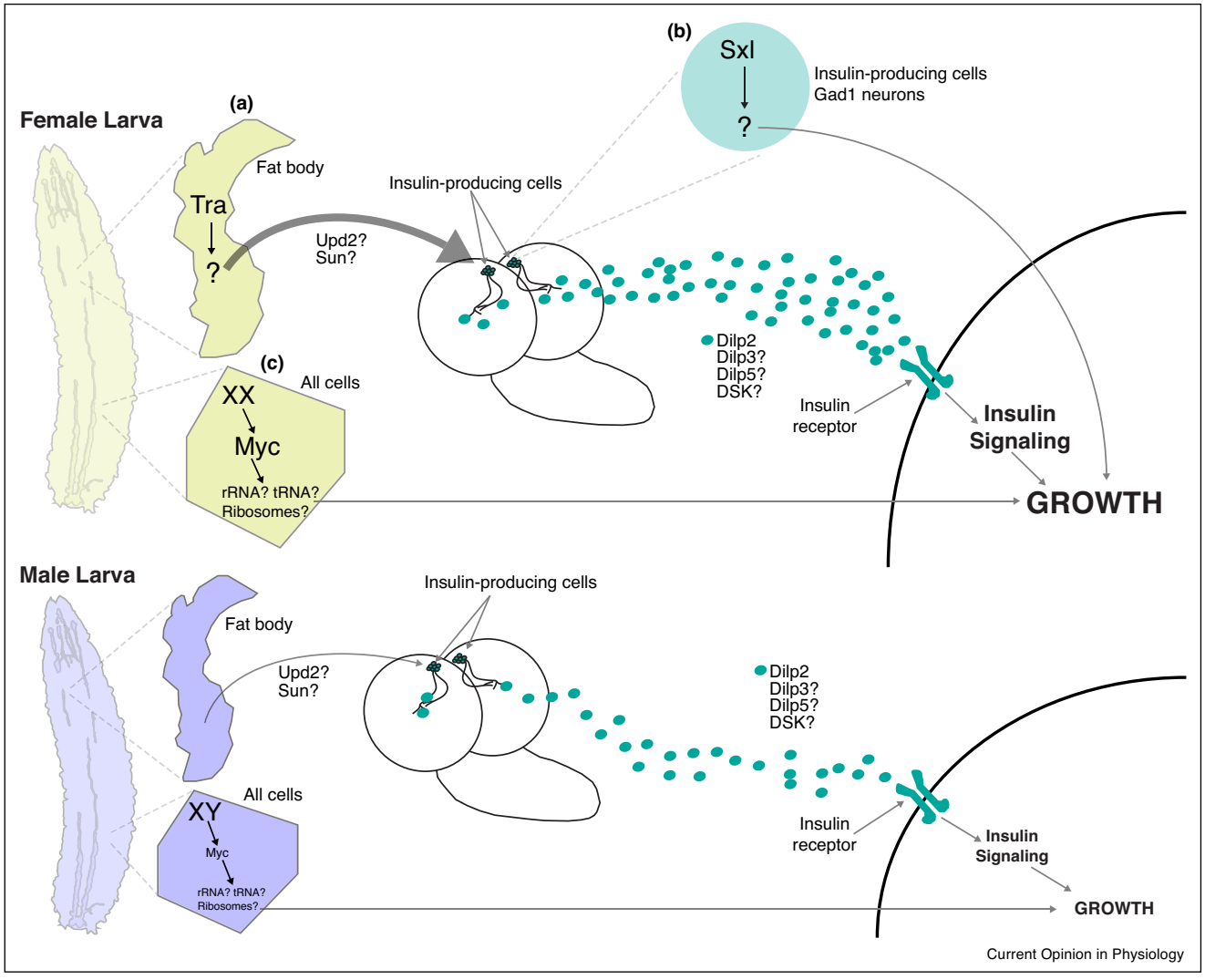
Other than IIS, several growth and signaling pathways have recently been found to have sex-biased effects on development and physiology. For example, reduced levels of potent growth regulator Myc in males likely contributes to their decreased larval weight, as increasing the copy number of Myc in males increases body size, whereas decreased Myc copy number reduces female larval weight [63•]. Similarly, the transforming growth factor-β (TGF-β) and epidermal growth factor receptor (EGFR) signaling pathways have sex-limited effects on wing shape and size [64•], and the Toll pathway is sex-specifically regulated both under normal conditions and in response to infection with Gram-negative bacteria [65••]. Future studies will be important not only to identify growth and signaling pathways with sex-biased effects, but also to determine the developmental and physiological significance of this sex-specific regulation.

Physiology and metabolism in adult flies

Drosophila is an emerging model to study metabolic regulation and physiology [66,67], yet few studies include both males and females. In this section we will describe recent advances in our knowledge of male–female differences in adult metabolism and physiology. While many of these differences reflect mating-induced changes in females rather than sexual dimorphism in physiology and metabolism, this knowledge provides an essential starting point for future studies on the mechanisms underlying male–female differences in physiology and metabolism.

Males and females differ in many aspects of metabolism and physiology under homeostatic conditions (e.g. lipid metabolism [68]). One important factor that affects male–female differences in physiology and metabolism is the level of circulating hormones. For example, titers of the steroid hormone ecdysone are higher in mated females [69–71]. Recently, this increased ecdysone level was shown to play a critical role in establishing a 'female

Figure 2



Multiple mechanisms contribute to sex differences in larval growth in *Drosophila*. A working model to integrate findings from recent studies on sex differences in the regulation of larval growth. Several mechanisms have been identified, which we describe in detail. **(a)** Two recent studies identified a role for the insulin/insulin-like growth factor signaling pathway (IIS) in the regulation of sex differences in body size (sexual size dimorphism, SSD) [32,42••]. A comparison of *Drosophila* insulin-like peptide (Dilp) regulation between males and females suggest females have higher Dilp2 secretion than males, whereas males have higher levels of *dilp3* mRNA [42••]. This increased Dilp2 secretion may affect IIS, as elevated IIS activity was detected in late third instar larvae [42••], though not at earlier stages [33••,42••], supporting a model in which female larval growth may be increased due to elevated IIS activity. Interestingly, the sex of the fat body, as determined by sex determination gene *transformer* (*tra*), influenced the secretion of *Drosophila* insulin-like peptide 2 (Dilp2) from the IPCs in a non cell-autonomous manner [42••]. The fat-to-brain signal responsible for this sex-biased Dilp2 secretion remains unknown, though multiple fat-to-brain signals have been identified in other studies (e.g. *stunted* (*sun*) [57], *unpaired-2* (*upd2*) [55]). **(b)** A recent study proposed an additional mechanism for the regulation of sex differences in body size. In this model, the sex of the IPCs and Gad1 neurons, as determined by sex determination gene *Sex-lethal* (*Sxl*), controls male–female differences in larval weight [33••]. Interestingly, Sxls regulation of body size is independent of its main target gene *Tra*. Furthermore, although the IPCs are known to produce Dilp2, Dilp3, Dilp5 [40,41], null mutations in *dilp2* and mutants lacking *dilp1-5* do not completely abolish SSD [33••], suggesting additional IPC-derived factors, such as Drosulfakinin [133], may be involved. **(c)** One final study demonstrated that potent growth regulator Myc may play a role in the regulation of SSD [63•]. Normally, Myc mRNA levels are higher in females than in males [63•]. Interestingly, males carrying duplications spanning the Myc locus had a larger body size than control males, whereas females heterozygous for a Myc mutant allele were smaller than control females [63•]. Together, these results suggest that increased Myc levels in females promote an increased rate of larval growth, perhaps through changes to the levels of ribosomal RNA (rRNA), ribosome biogenesis, and transfer RNA (tRNA) [134,135].

metabolic state' in which females store increased levels of triglyceride and glycogen than males [72••]. This increased energy storage likely plays an important role in supporting the energetic demands of reproduction [72••], however, future studies will need to determine whether male–female differences in ecdysone titers and energy storage also exist independently of mating. In addition to modulating energy storage, ecdysone also regulates early female germline sexual differentiation [73•], somatic cyst stem cells in the male testis [73•], cell division in germline stem cells in the ovary [74], and the creation of sexually dimorphic neural circuits [75]. Given that ecdysone regulates diverse aspects of physiology and metabolism [76], more studies will be required to identify additional phenotypes associated with male–female differences in levels of ecdysone, and other circulating factors such as JH, which modulates lipid metabolism in the enterocytes of mated females [10•].

Recent studies have also made progress in understanding male–female differences in metabolism in response to stress and aging. For example, males and females differ in adaptation to oxidative stress induced by hydrogen peroxide (H_2O_2) [77••]. In females, pre-treatment with low doses of H_2O_2 before challenge with higher H_2O_2 doses promotes survival; males show no survival benefit after H_2O_2 pre-treatment. Interestingly, sex-specific expression of a mitochondrial Lon protease isoform is critical for this female-specific adaptation to H_2O_2-induced stress [77••]. In addition to mitochondrial Lon protease, there is also sex-specific induction of the 20S proteasome during adaptation to H_2O_2-induced stress [78], and sex-specific sensitivity to genetic variation in the NADP(H) enzyme network [79]. Sex-specific effects of metabolic regulation on lifespan have been reported for mitochondrial thioredoxin reductase 2 [80], cytosolic copper/zinc superoxide dismutase [81,82], and DNA repair genes [83], and one recent study described male–female differences in feeding behavior, stress resistance and lifespan in response to high sugar feeding [84]. Together with the sex-specific effects of genes on stress responses and longevity identified using quantitative genetic approaches [85–90], these recent studies underline the importance of examining both sexes in future studies of metabolism, stress responses, and aging.

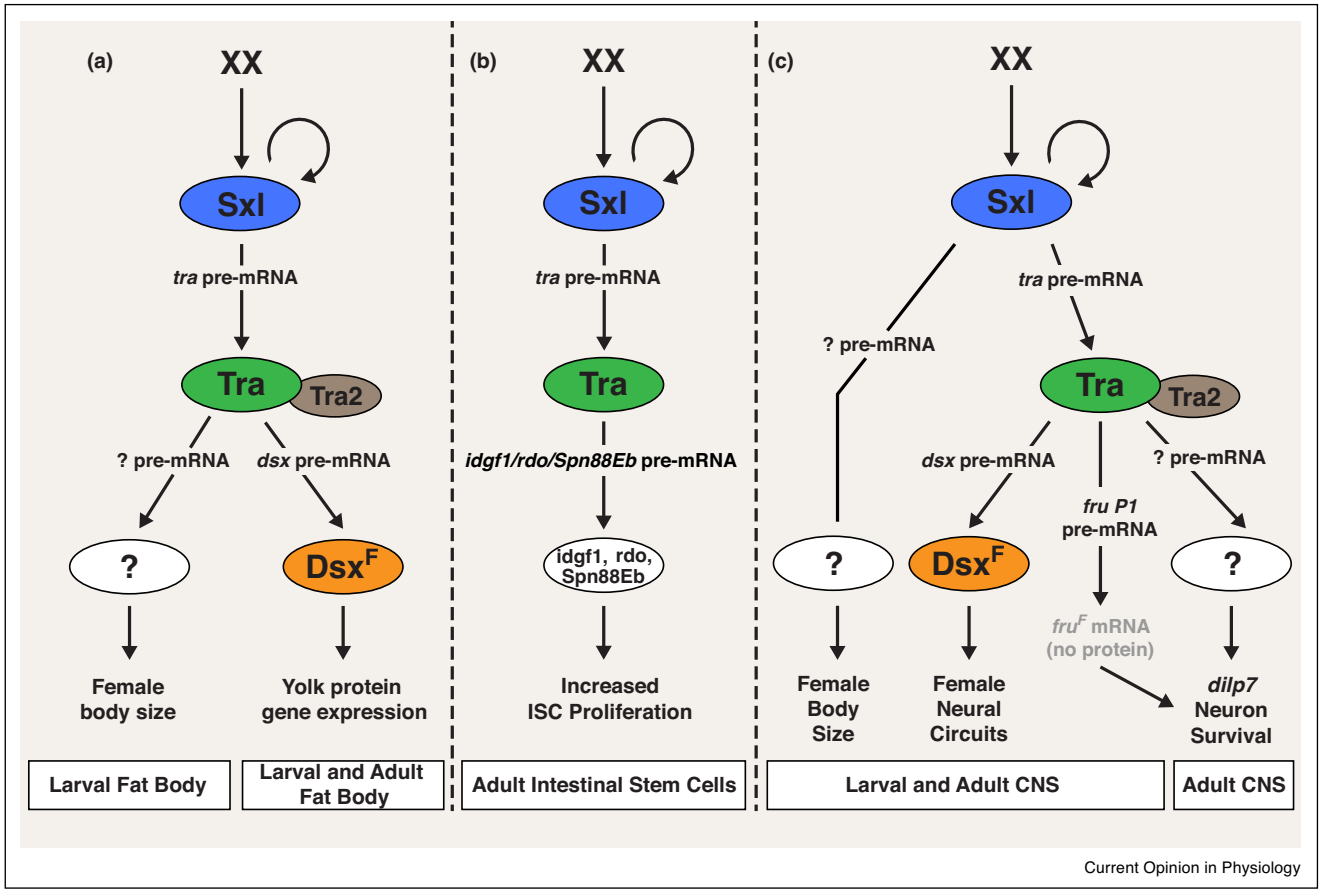
Although the studies described above focus on the genetic, molecular, and biochemical mechanisms underlying sex differences in metabolism, it is important to note that sexual dimorphism exists in behaviors that modulate physiology and metabolism. For example, males and mated females flies differ in sleep [91•,92,93], food intake [72••], and food preferences [94,95]. Future studies will provide a more complete understanding of sex differences in physiology by addressing how sex differences in behavior impact male–female differences in metabolism.

New insights into *Drosophila* sex determination: studies in females lead the way

Two X chromosomes in female flies triggers the production of an X-derived protein called Sex-lethal (Sxl) [96–98]. Sxl is a splicing factor that introduces a sex-specific splice into the pre-mRNA of its main downstream target, *transformer* (*tra*); this splicing allows a functional Tra protein to be produced in females [99–101]. Tra, also a splicing factor, interacts with its co-factor *transformer-2* (*tra2*) to bind the pre-mRNA of its target genes *doublesex* (*dsx*) and *fruitless* (*fru*) [102–105]. Tra-dependent splicing of *dsx* pre-mRNA produces a female-specific isoform called Dsx^F. Tra-dependent splicing of *fru* pre-mRNA introduces a stop codon into P1 promoter-derived transcripts, thus no Fru P1 proteins are produced in females. In males, one X chromosome means that no Sxl is produced, and functional Tra protein is absent. Without Tra, *dsx* and *fru* P1-derived transcripts undergo default splicing to produce the male-specific isoforms of each gene, Dsx^M and Fru^M, respectively. Together, these genes explain many aspects of sexual development, reproduction, and behavior [1,5,7,106–110]. In this section, we will highlight new insights into the canonical sex determination pathway in *Drosophila* from recent studies on female development and physiology (Figure 3).

The prevailing model of *Drosophila* sex determination suggests the primary function of Tra is to ensure the appropriate sex-specific splicing of *dsx* and *fru* P1-derived transcripts. The regulation of sexual identity by *dsx* and *fru* has therefore been an intensive area of research, yielding important insights into sexual development and reproduction [7,107–120]. Over the past two years, studies on sex differences in development and physiology have identified additional Tra-regulated phenotypes. For example, larval size and adult weight are both reduced in females lacking Tra [42••,63•], while loss of Tra in males has no effect [42••]. Although Tra may regulate body size partly via cell-autonomous effects on cell size, Tra function in the fat body also plays a key role in mediating the effects of Tra on body size, as rescuing Tra only in the fat body restored a normal body size to *tra* mutant females [42••]. Interestingly, Tras effects on larval body size are independent of its only known targets, *dsx* and *fru*, identifying a previously unrecognized branch of the sex determination pathway that is Tra-dependent, but *dsx*-independent and *fru*-independent [42••] (Figure 3). Instead, the *tra*-induced reduction in female body size may be due to changes in the IIS pathway, as females lacking fat body *tra2* have reduced levels of Dilp2 secretion from the IPCs, and genetically augmenting IIS activity restores a normal body size to *tra* mutant females [42••] (Figure 2). However, the molecular mechanisms underlying Tras regulation of Dilp2 secretion are unclear, emphasizing a need for more knowledge on the molecular mechanisms linking Tra and IIS.

Figure 3



New insights into the *Drosophila* sex determination pathway. According to the prevailing view of *Drosophila* sex determination, female sex is specified by the presence of two X chromosomes, which triggers the production of an X-derived protein called Sex-lethal (Sxl). Sxl is a splicing factor that introduces a sex-specific splice into the pre-mRNA of its main downstream target, *transformer* (*tra*); this binding allows a functional Tra protein to be produced in females. Tra, also a splicing factor, binds to the pre-mRNA of its target genes *doublesex* (*dsx*) and *fruitless* (*fru*). Tra-dependent splicing of *dsx* pre-mRNA produces a female-specific isoform called Dsx^F. Tra-dependent splicing of *fru* pre-mRNA introduces a stop codon into P1 promoter-derived transcripts, thus no Fru P1 proteins are produced in females. Recent studies on development and physiology have expanded our knowledge of the sex determination pathway by identifying additional downstream branches of the pathway, and by identifying context-dependent effects of these pathways in different tissues, and at different times during development. For example, in addition to the canonical sex determination pathway that operates in the larval and adult fat bodies **(a)**, there is an additional branch of the sex determination pathway that is Tra-dependent, but *dsx*-independent, that regulates body size in female larvae [42••]. Tras regulation of body size via this newly identified branch depends on Tras binding partner *transformer2* (*tra2*) [42••]. Future studies will need to identify downstream targets of Tra that mediate its effects on larval growth. **(b)** In the intestinal stem cells (ISCs) this Tra-dependent, *dsx*-independent, branch of the sex determination pathway promotes increased ISC proliferation; however, this function of Tra does not require *tra2* [9••]. Candidate Tra targets that affect ISC proliferation include *imaginal disc growth factor 1 (idgf1)*, *reduced ocelli* (*rdo*), and *Serpin 88Eb* (*Spn88Eb*) [9••]. **(c)** In the larval and adult central nervous systems (CNS), Tra and Tra2 specify female neural circuits via regulation of *dsx* and *fru* pre-mRNA in the canonical sex determination pathway, as previously described. Interestingly, Tra may regulate the survival of female-specific *Drosophila insulin-like peptide 7* (*dilp7*)-expressing neurons in adults through both canonical, and non-canonical pathways [121,122]. In adults and larvae, two recent studies have identified a Sxl-dependent, but Tra-independent, branch of the pathway that functions in subsets of neurons to create sex differences in physiology [33••,123]. For example, Sxl function in IPCs and Gad1 neurons plays a critical role in creating sex differences in larval weight [33••].

While knowledge of the downstream effectors of Tra is less developed with respect to its regulation of body size, more progress has been made in identifying the genes downstream of this Tra-dependent, *dsx*-independent and *fru*-independent, pathway in ISCs. Hudry *et al.* (2016) showed that sexual dimorphism in ISC proliferation in the *Drosophila* intestine is regulated by *Sxl* and *tra* independently of *fru* and *dsx* [9••]. Significantly, this study identified several new Tra-regulated genes that reproduce Tras effects on ISC proliferation: *reduced ocelli* (*rdo*), *imaginal disc growth factor 1* (*idgf1*), and *Serpin 88Eb* (*Spn88Eb*) [9••] (Figure 3). Although the molecular mechanism underlying Tras regulation of these putative target genes remains unclear, it is interesting to note that Tras

effects on ISC proliferation and body size occur via distinct molecular mechanisms — Tra regulates body size together with its binding partner *tra2* [42••], whereas sex differences in ISC proliferation are *tra2*-independent [9••]. Interestingly, Tra may act through both canonical and non-canonical mechanisms in the central nervous system (CNS) to regulate the survival of female-specific *dilp7* neurons [121,122]. Future studies will be important to elucidate how Tra acquires this cell-specific and tissue-specific activity at the molecular level.

In addition to Tra, novel insights into the regulation of Sxl, and its effects on development and physiology, have recently been published. For example, loss of *Sxl* in postmitotic neurons abolished SSD [33••], an effect that was mediated by *Sxl* function in at least two subsets of neurons, the IPCs and Gad1 neurons (Figure 2), but potentially independently of IIS. Interestingly, Sxls regulation of SSD was not Tra-dependent, corroborating a previous report [123] of a Sxl-dependent, but Tra-independent, branch of the sex determination pathway in the CNS. Thus Sxl and Tra act in specific tissues to influence SSD via regulation of non-canonical target genes, although their effects differ in magnitude: loss of Tra in the fat body reduces SSD [42••], whereas reduced Sxl function in neurons abolishes SSD [33••]. Future studies will be required to identify Sxl targets in addition to Tra that mediate its effects on SSD, and to elucidate the mechanisms underlying the regulation of SSD by both Sxl and Tra, especially in light of data suggesting the presence of feedback loops in the sex determination pathway [107,124]. Further, since *Sxl* is responsible for the regulation of both dosage compensation and sex determination, it will be important to understand how changes to *Sxl* affect neuronal development, connectivity and function in the IPCs and Gad1 neurons, and to rule out any adverse effects of changes to the dosage compensation machinery in these neurons. Finally, more work will be needed to explore a role for *Sxl* in other aspects of development and physiology. For example, several studies recently identified *spenito* (*nito*) as a novel regulator of *Sxl* auto-regulation [125–127]. In females, loss of *nito* causes masculinization of female structures by disrupting the transfer of an N^6-methyladenosine (m^6A) modification to *Sxl* pre-mRNA that is required for *Sxls* female-specific alternative splicing [125,126]. Interestingly, *nito* plays a key role in maintaining triglyceride homeostasis in *Drosophila* larvae [128•]. Although the larvae in the *nito* study were not sexed, future studies will determine whether *nito* affects sex differences in triglyceride homeostasis in part through its interaction with *Sxl*.

Beyond *Sxl* and *tra*, *tra2*, *dsx* and *fru* also have unexplored roles in the regulation of development and physiology. For example, *tra2* affects the regulation of triglyceride storage in adults [129], *dsx* regulates cell size and mRNA levels of many circulating factors known to affect development and physiology [42••,107], and the activity of *fru* neurons has been implicated in the regulation of fat storage [130]. Thus, studies of development and physiology are rapidly identifying new roles for established sex determination genes. In addition, studies on factors that regulate development and physiology have provided new insights into *Drosophila* sex determination. For example, steroid hormone ecdysone affects sex determination via regulation of the *let-7-C* micro-RNA cluster [73•], and *Chronologically inappropriate morphogenesis* (*Chinmo*) affects sexual identity in the *Drosophila* testis via regulation of sex determination genes *dsx* and *tra* [131,132]. Although more studies are needed to identify additional genes and pathways that modulate the regulation of sex determination genes, these studies are likely to yield exciting new insights into sex determination in *Drosophila*. Together with studies to increase knowledge of sex differences in development and physiology, a deeper understanding of sex determination mechanisms will ensure *Drosophila* remains a leading model for studies of sex differences in development and physiology.

Conflict of interest statement

Nothing declared.

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